Gestational trophoblastic tumours and non-neoplastic trophoblastic lesions:

morphology and immunocytochemistry to refine the diagnosis

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Abstract

Gestational trophoblastic disease (GTD) is generally subclassified into hydatidiform moles (HM), Gestational trophoblastic tumours (GTTs) and non-neoplastic trophoblastic lesions. GTT represent a spectrum of neoplasms that originate from the extravillous trophoblast and have variable malignant potential. These include choriocarcinoma (CC), Placental site trophoblastic tumour (PSTT) and Epitheloid trophoblastic tumour (ETT). Among tumour like conditions exaggerated placental site reaction (EPSR) and placental site nodule (s)/ plaque (s) are included. The morphological appearances of GTT can be mimicked both by non-malignant tumour-like conditions and also non-gestational tumours with trophoblastic differentiation, adding to the diagnostic dilemma of these already rare tumours. GTT have a favourable prognosis and better response to specific chemotherapeutic regimens when compared to non-gestational malignant genital tract neoplasms, and thus their recognition is important. This article discusses the morphological appearances and immunocytochemistry of these rare tumours along with recent developments in the form of targeted immunotherapy.

Key words: choriocarcinoma, placental site trophoblastic tumour, epithelioid trophoblastic tumour, exaggerated placental site reaction, placental site nodule, immunocytochemistry.

Introduction

Gestational trophoblastic tumours (GTT) represent a wide spectrum of neoplasms that originate from the extravillous trophoblast and have variable malignant potential. Most originate in the uterus and a few are encountered at other locations, both within the female genital tract and at other sites (eg lung). The WHO 2014 classification of GTT includes choriocarcinoma (CC), Placental site trophoblastic tumour (PSTT) and Epitheloid trophoblastic tumour (ETT); however, the clinical classification of GTT includes invasive hydatidiform moles (IM) and molar metastasis as malignant disorders requiring chemotherapy. Among tumour like conditions, exaggerated placental site reaction (EPSR) and placental site nodule (s)/ plaque (s) (PSN) are included.

The morphological appearances of GTT can be mimicked both by non-malignant tumour-like conditions and also by non-gestational tumours showing trophoblastic differentiation, adding to the diagnostic difficulty of these already rare tumours. In addition, specific classification of GTT may be difficult in some cases as histological features may have been altered by treatment, and some lesions may demonstrate mixed phenotypic morphology. The appropriate diagnosis of GTD/GTT is therefore based on a combination of clinical, biochemical, genetic and histopathological findings. GTT have a favourable prognosis and better response to specific chemotherapeutic regimens when compared to non-gestational malignant genital tract neoplasms, and thus their recognition is important.

<A>Gestational trophoblastic tumours

<B>Choriocarcinoma

<C>Introduction

Choriocarcinoma (CC) is the most common malignant gestational trophoblastic

neoplasm with differentiation towards the phenotype of villous trophoblast, including

cytotrophoblast, intermediate trophoblast and syncytiotrophoblast. It occurs primarily in

patients of reproductive age (mean 30 years) although rare cases of gestational CC

have been reported in post-menopausal patients after long latency (1). In Western

countries gestational CC occurs in approximately 1:20,000–50,000 pregnancies (2) but

the frequency varies geographically. Incidence is higher in Southeast Asia and Japan

with rates of 9.2 and 3.3 per 40,000 pregnancies, respectively (3).

Most CC are preceded by an abnormal pregnancy (after CHM, PHM) although some

occur following normal term/preterm pregnancies, ectopic pregnancy or spontaneous

miscarriage. The incidence of CC after CHM (about 1 in 50) is about a thousand times

greater than after a normal pregnancy (about 1 in 50,000). Incidence following PHM

varies from 1:1000 to 1:200. The proportion of CCs preceded by moles in reported

series varies between 40-80%, but this is probably an underestimate due to under-

recognition of early complete moles in the historical literature (4). It is generally

assumed that the immediate antecedent pregnancy is responsible for the tumour and although in most cases it may be so, it is not necessarily the case (5,6).

<C>Clinical features

Vaginal bleeding and marked elevation (>10,000 mIU/L) of serum human chorionic gonadotropin (hCG) are the characteristic clinical findings; however some cases may present with a metastatic disease and subsequent diagnosis of the primary tumour. A variety of clinical presentations have been described ranging from cervical or vaginal nodules to pulmonary, central nervous system and liver metastases with secondary cor-pulmonale or intracerebral haemorrhage (7,8). Melena or haematuria secondary to involvement of gastrointestinal or renal tract have been reported, and metastases to other even more unusual sites (extraocular muscle, skeletal muscle, spleen) have been described. Indeed, any unusual clinical symptoms/signs in a woman of childbearing age should raise the possibility of CC and failure to recognise has even resulted in the development of CC in immunosuppressed organ recipients (9). In rare cases the primary tumour cannot be discovered and is assumed to have undergone spontaneous regression.

The tumour is mainly uterine but extrauterine sites such as the fallopian tube and ovary have also been reported (10, 11). Approximately two thirds of these patients present with symptoms of vaginal bleeding and abdominal pain with a positive pregnancy test, clinically identical to a routine ectopic pregnancy. Others may present with an apparent ovarian mass suggesting an ovarian tumour or with metastatic disease. In contrast to ectopic tubal CC, primary CC in other organs including lung, breast, stomach, intestine,

liver, adrenal, pancreas, bladder and kidney are likely to represent non-gestational carcinomas with aberrant trophoblastic differentiation, which can now be confirmed by molecular genotyping (11).

Morphology

Typically the tumour appears as bulky single to multiple dark red masses with extensive haemorrhage and variable amount of necrosis. With advancements in imaging techniques and better follow-up protocols available for monitoring of molar pregnancies, many smaller tumours are being increasingly detected.

Microscopically, the tumour shows variable degrees of haemorrhage and necrosis, and a typical characteristic bilaminar pattern reminiscent of early villous trophoblast. The neoplastic cells are an admixture of syncytiotrophoblast, intermediate trophoblast and cytotrophoblast-like morphology. The bi/ tri-phasic pattern is easily identifiable but in some cases most of the tumour is either predominantly syncytiotrophoblastic or predominantly cytotrophoblastic. Blood–lakes, pseudo vascular channels and geographic necrosis are a frequent accompaniment. Marked nuclear pleomorphism and numerous sometimes abnormal, mitotic figures are present (Figure 1).

Cases showing characteristic morphology are fairly easy to diagnose. However certain morphological variants pose diagnostic difficulties. The *monomorphic variant* shows mainly mononuclear cells arranged in cohesive sheets resembling poorly differentiated carcinoma. Syncytiotrophoblast may be inconspicuous. This appearance is commonly

seen in cases with previous chemotherapy. *Paucicellular variant* containing scanty

viable tumour, at the periphery of the nodule and within vascular spaces, with large

areas of haemorrhage and necrosis may also be a diagnostic challenge. Both these

variants may require extensive sampling, examination at levels or use of beta-hCG

immunocytochemistry to highlight the second component.

In general, GTT arise from extravillous trophoblast and hence choriocarcinoma is not

associated with chorionic villi with the exception of intraplacental CC that arises in a

term placenta. It is well-recognized that CC may follow, and be genetically derived from,

apparently non-molar pregnancies, and when occurring in the third trimester placenta is

termed intraplacental CC. On gross examination no specific features may be seen and

foci resembling either placental infarcts or thrombi are present which may be missed on

initial examination. In many cases, this may be a retrospective diagnoses. On

histological examination, apparently normal chorionic villi are surrounded by a highly

pleomorphic, bilaminar trophoblast proliferation, which often locally fills the intervillous

space and it is associated with fibrin deposition (12).

Molecular genetic testing has confirmed that both maternal and fetal metastatic disease

may be seen from the intraplacental choriocarcinoma and maternal choriocarcioma is

reported in approximately 50% of the cases (13).

It has been suggested that extensive trophoblast pleomorphism might represent

coexistence of CHM and CC (14). However, until further data are available, the

consensus is that CC should not be diagnosed regardless of the degree of trophoblast pleomorphism if there is CHM with chorionic villi present. Accordingly, 'CHM with CC' was not included as a category in the most recent WHO Classification (15).

<C>Immunocytochemistry

A panel of immunomarkers is used for establishing the diagnosis of trophoblastic tumour as no single marker is sensitive or specific. Several well-established trophoblastic markers include hPL, hCG, Mel-CAM (CD146), p63, MUC-4, HLA-G, cyto-keratin18, and inhibin-A. As choriocarcinoma contains a variable admixture of cytotrophoblast, intermediate and syncytio-trophoblast; all of these markers are positive to a variable extent in this tumour (16, 17, 18)

GATA-3 is a recent addition with around 80% of CC showing nuclear positivity of variable intensity (19). Various studies have validated use of GATA-3 as a trophoblastic marker. One study claims that HSD3B1 is highly specific and sensitive marker expressed in all types of trophoblastic lesions but there are no subsequent studies on this marker (20) and this antibody is not available for commercial use (Figure 2).

SALL-4 expression has been described to help differentiate CC from PSTT/ETT in one study including 19 CC and 10 PSTT/ETT. 100% of CC were positive for SALL-4 while none of the PSTT/ETT tumours showed expression (21) but no further studies validated the use of SALL-4 in GTD have been conducted.

Whilst a range of molecular findings have been described in CC, no distinctive diagnostically or prognostic ally useful molecular signature exist. Studies have often been performed in CC cell lines and overexpression of VEGF (22) and downregulation

of ASPP2 (23) have been reported but at present no consistent or reliable alterations have been identified for clinical use.

Prognosis and treatment

Chemotherapy is the mainstay of treatment for CC, with overall cure rates of >90% as these are highly chemosensitive tumours.

Intermediate trophoblast lesions

Introduction

Intermediate trophoblast is derived predominantly from non-villous trophoblast and is mainly associated with normal placental implantation site and the chorionic membranes, from which a range of trophoblastic lesions arise, namely; exaggerated placental site reaction (EPSR), placental site nodule/plaque (PSN), placental site trophoblastic tumour (PSTT), and epithelioid trophoblastic tumour (ETT). EPSR and PSTT most closely recapitulate implantation site trophoblast whereas PSN and ETT more closely resemble chorionic-type intermediate trophoblast. Conceptually, EPSR and PSN can be thought as the non-neoplastic counterparts of PSTT and ETT respectively, the latter two representing distinct subtypes of malignant GTT that although phenotypically and immunohistochemically distinct, show overlap in their clinical and pathological features (24).

Placental site trophoblastic tumour

PSTT is derived from implantation site intermediate trophoblast (ISIT). These were originally thought to be benign neoplasms with favourable prognosis and hence were designated as 'trophoblastic pseudotumor' (25). However, further evidence has shown that PSTT can behave aggressively, exhibiting local invasion and even metastatic spread. Thus, the term 'placental site trophoblastic tumour' was introduced based on its clinical behaviour and its phenotypic similarity to the implantation site trophoblast (26).

<C>Clinical features

Most patients are of child bearing (mean 31) age and less commonly peri/postmenopausal (27), present with vaginal bleeding, uterine enlargement or amenorrhea (27). Serum hCG levels are almost always increased, but are usually far less elevated (average around 700 mIU/ml) than in CC, and may include specific hCG subtypes, particularly its free-beta fraction (28, 29). A few patients may present extensive local spread or metastases involving lungs, peritoneum, liver, pancreas and brain. Rarely, PSTT may be associated with paraneoplastic syndromes or secondary manifestations, including nephrotic syndrome due to glomerular intracapillary deposits of immunoglobulin and fibrin and virilisation due to hormonal stimulation (30, 31). The interval between last known antecedent pregnancy and clinical presentation may range from months to several years (average three years), in contrast to the usually short interval period in CC (32). PSTT can genetically arise from both molar and non-molar conceptions, with an unexplained but striking predominance of female conceptions (33).

<C>Pathological features

PSTT form a uterine mass that can be small (1 to 2cm) or may replace most of the uterus. These are well or poorly circumscribed with a soft, white to tan to yellow cut surface. Interspersed areas of necrosis and haemorrhage may be seen. A few may be grossly mistaken for fibroids. Deep myometrial invasion (in 50% cases) and less commonly perforation and extension to adjacent organs (10% cases) can be a feature.

On microscopic examination, PSTT are characterised by a diffuse and infiltrative growth of monomorphic intermediate trophoblast arranged in sheets and cords. These are typically seen to separate myometrial fibres ("splitting") and replace uterine blood vessel walls with extensive fibrinoid deposition, reminiscent of the normal implantation site. This typical pattern of vascular invasion, with overall maintenance of vascular architecture, is characteristic of PSTT and destructive vascular invasion with haemorrhage is much less frequent than that seen in CC. Most cells are mononuclear and polygonal with abundant amphophilic or eosinophilic cytoplasm, but cells with clear cytoplasm may also be found. Many have a single irregular hyperchromatic nucleus but binucleated and rarely multinucleated (syncytiotrophoblast-like giant) cells are present. Eosinophilic hyaline globules, nuclear pseudo-inclusions and nuclear grooves can be a feature. The mitotic rate is typically low (2-4/ 10 HPFs) in most tumours but atypical mitotic figures are not uncommon (Figure 3). In long-standing or post-chemotherapy tumours, secondary dystrophic calcification may also be identified, with viable tumour cells only identified at the periphery close to vessels. Rarely tumours originate at extrauterine sites, including fallopian tube and ovary (34,35).

<C>Immunohistochemical and other ancillary features

Immunohistochemical stains often provide useful diagnostic information in PSTT. There is widespread expression of cytokeratin (AE1/3, Cam5.2 and CK18), CD10, HLA-G and GATA-3 in keeping with trophoblast lineage. Most cell express hPL, MUC-4 and Mel-CAM while occasional may be positive for PLAP and p63. hCG and inhibin expression is limited to multinucleated (syncytiotrophoblast-like giant) cells (Figure 4). Proliferation index is usually low as compared to CC (10-30%).

Although electron microscopic features of GTTs are described, with ultrastrucural features of trophoblast similar to normal pregnancy, this technique does not currently have a role in clinical diagnosis (36).

<C>Genetics

In general, compared to CC, most PSTT are diploid (37). There is a confirmed and marked female predominance of these GTTs, which remains unexplained (33) but genetic studies have otherwise demonstrated no consistent chromosomal loss or gains. Molecular genotyping is used to determine causative pregnancy in these tumours as it has major prognostic implications (detailed in article by Fisher on Molecular genotyping, elsewhere in this issue).

<C>Prognosis and treatment

These have been discussed in clinical article by Seckl elsewhere in this issue.

<B>Epithelioid trophoblastic tumour

This more recently described and rarest form of GTT was initially termed "atypical choriocarcinoma", with subsequent recognition as a distinct entity (1994) (38).

<C>Clinical features

Patients are usually in their reproductive years (mean 36.1 years) and previous history includes a full term delivery (70%), spontaneous miscarriage (15%) or HM (15%). Interval between previous gestation and diagnosis of an ETT may be quite long (average 6.2 years) and is slightly longer than that seen in PSTT (39).A few cases have however been reported in pre/ post-menopausal females. The most common presenting complaint is abnormal vaginal bleeding, amenorrhoea or rarely patients may present with metastases (40). As with PSTT, serum hCG is almost always elevated at the time of diagnosis, but levels are very low (<2500mIU/L) in most cases.

<C>Pathological features

ETT may be located with equal frequency in the lower uterine segment or cervix (50% cases) as in the uterine corpus. It presents as solid/ cystic, white or tan nodules with frequent areas of necrosis and haemorrhage. Tumour size may range from 5-50mm with macroscopic involvement of deep myometrium and adjacent structures. On microscopy, these are expansile circumscribed tumours with 'carcinoma-like'

architecture; comprising monotonous population of mononucleated intermediate trophoblast arranged in nests, cords and islands (39,40). The tumour cells have eosinophilic or clear cytoplasm, well-defined cell membranes and round uniform nuclei and are smaller and show less nuclear pleomorphism than PSTT cells. Mitotic count ranges from 0 to 9/ 10 HPFs but mitoses up to 48/10HPFs have been reported (41). Hyaline eosinophilic material, composed of type IV collagen and fibronectin, is usually seen intimately admixed with tumour cells and forming coalescing nodules. This dense eosinophilic material may resemble keratin and hence closely, mimics squamous cell carcinoma of cervix. Necrosis, imparting a "geographic" appearance and dystrophic calcification may also be noted. A peritumoral lymphocytic infiltrate is seen in approximately half of cases (Figure 5). It should be noted that other types of GTT when examined postchemotherapy may have a morphological phenotype that overlaps with ETT often showing extensive necrosis with only scanty viable peripheral mononuclear tumour cells, with low Ki67 index but p63 positivity (42).

<C>Immunohistochemical features

Tumour cells express cytokeratin (CK18, AE1/3, Cam5.2), CD10, HLA-G and GATA-3 in keeping with trophoblast lineage. EMA, Cyclin-E, p63, inhibin and PLAP are usually diffusely expressed, whereas Mel-CAM, hCG and hPL expression is weak and focal. p63 is reliably positive in ETT and is a useful marker in the differential diagnosis with other malignant trophoblastic tumours (34, 43) There is increased expression of cyclin E when compared to PSN, but of note PSTT also shows cyclin E positivity (44).

<C>Genetics

These tumours do not have characteristic molecular genetic findings, although their

gestational origin and female preponderance has been confirmed (45, 46)

<C>Prognosis and treatment

Discussed in clinical article by Seckl elsewhere in this issue.

<A>Tumour-like conditions

<B>Exaggerated placental site reaction (EPSR)

This lesion does not represent a trophoblastic neoplasm but rather the residual

morphologic expression of the implantation site reaction following a recent gestation

which may be molar or non-molar. EPSR are usually diagnosed following curettage

when evaluating post-conception vaginal bleeding. On microscopic examination,

fragments of endomyometrium are infiltrated by individual and nests of intermediate

trophoblast, with organisation and morphological characteristics of normal implantation

site. There is neither confluent growth nor destruction of the underlying myometrial

architecture (Figure 7 A-D). The presence of evenly spaced implantation site-type

multinucleate trophoblast giant cells is a useful diagnostic feature (47, 48).

In some cases the diagnosis of PSTT may be raised in a post-conception curettage

specimen in which retained chorionic villi are also present in association with a florid

implantation site reaction. In such cases, the presence of villi largely excludes the diagnosis of PSTT. Definitive distinction from PSTT is overall based on both clinical and morphological findings and immunohistochemical profile. Specifically, EPSR demonstrates no mitotic activity and an extremely low (<2%) proliferation index (47). Double MelCAM/ Ki-67 stain may be used to highlight proliferation with implantation site trophoblast and is very useful to distinguish from proliferation within inflammatory or glandular cells ((Figure 7 E-F). Although not required in clinical practice, it has also been demonstrated that EPSR are genetically different from PSTT, since they have the normal expected ratio of male and female trophoblast derived cells versus the documented female predominance in PSTT (48).

It should be also noted that, florid implantation site reaction is well-described in association with CHM and should not in this context be regarded as EPSR or PSTT.

<B>Placental site nodule (PSN)/atypical PSN (aPSN)

<C>Clinical features

PSNs are reported in the reproductive age group and are derived from intermediate trophoblast of chorion leave type. It is often an incidental finding in endometrial curettings performed for bleeding abnormalities, abnormal cervical smear or other indications. A significant association is seen with a history of a tubal ligation (49) and many have a history of therapeutic abortion or caesarean section. *Although this likely represents remnants of a previous, sometimes unrecognized pregnancy; in some cases*

antecedent pregnancy has been reported to have occurred as many as 9 years before

the diagnosis PSN is not proliferative and is therefore not associated with elevated

hCG levels (49-51, 52).

<C>Pathological features

Most PSN's are seen in endometrium, lower uterine segment, and cervix with rare

cases occurring in fallopian tube, broad ligament and ovary. It may be seen as a single

or multiple lesions, usually yellow to tan in colour and ranges from 1 to 14 mm (average

2mm). Histological examination reveals small, well-circumscribed nodules composed of

intermediate trophoblast of chorionic type with central hyalinisation. The lesions are

paucicellular with mainly single cells or cells arranged in small clusters and cords.

Occasional multinucleated cells are present. The mononuclear cells have small uniform

nuclei and abundant eosinophilic to clear cytoplasm. Mitotic figures and necrosis are not

seen in PSN (Figure 8 A-C)PSNs maintain the lobular architecture and the diagnosis is

often straightforward. If cellular or with unusual features, curettage specimens should be

interpreted in the light of the clinical/radiologic findings, since in the presence of an

image-identifiable lesion, sampling at the periphery of an ETT or less often a PSST may

erroneously suggest a PSN.

It is now recognised that rare lesions may be encountered with histopathological

features intermediate between typical PSN and PSTT/ETT, and these have been

termed as Atypical Placental Site Nodule (APSN).

There are no well-established objective histological criteria to definitively distinguish

APSN as an entity, other than general agreement that they represent lesions which

appear 'atypical' to histopathologists experienced in this area. The lesions are generally larger in size than typical PSN (most PSN's have been described as <5mm and APSN's as > 5mm in size.), with higher cellularity, more cohesive nests and cords of viable trophoblastic cells and increased proliferation (>5%) compared to typical PSNs (Figure 9) but exact criteria for distinguishing typical from atypical for these variables have not been established (49- 51).

Immunohistochemical features

This lesion, like other trophoblastic lesions, is positive for anti-cytokeratin antibodies and are invariably positive for alpha-inhibin, EMA and GATA-3. Most lesional cells express PLAP and p63 (Figure 8 D) while a small number are positive for hPL and MelCam (CD146) (49- 51). The trophoblastic cells in PSNs exhibit low proliferative activity with a Ki-67 labelling index of about 5%, similar to that in the intermediate trophoblastic cells in the chorion laeve while for APSN it is usually <10%. CyclinE-positivity has been described in some APSN and as in ETT.

Prognosis and treatment

Although typically PSNs do not require follow up, limited data regarding APSN have shown an association with malignant GTT in 15% cases. These developed either concurrently or within 16 months of the APSN diagnosis (53). Furthermore, examples of PSTT/ETT in hysterectomy specimens in which morphological areas of both PSN and PSTT/ETT are present have also been reported (54).

In addition to the data available from the largest series to date, isolated cases of transformation of PSN to a coexisting ETT and PSTT and another case, in which transformation of PSN to ETT with pelvic and lung metastasis has been reported. In view of these recent updates, we advocate thorough radiologic investigations to exclude an underlying mass lesion and follow up of patients diagnosed with APSN at our centre. hCG appears to be an unreliable marker for early detection of development of malignant GTD in patients with APSN, although studies specifically examining the role of free-β hCG, which has been reported as a more useful marker for PSTT are not available.

<B>Mixed gestational trophoblastic tumours

Although GTT are generally described as clinically and pathologically distinct entities it has become increasingly recognised that tumours with morphological and immunophenotype of CC and /or PSTT/ETT can exist (55). Similarly, whilst PSTT and ETT are referred as distinct entities, mixed tumours with both phenotypes have been well described (39,43)

Distinction of CC from PSTT/ETT is usually straightforward based on clinical features and serum hCG concentrations. If pre-chemotherapy specimens are sampled, histological examination demonstrates the distinctive features as described above. Specimens of CC examined post-chemotherapy may demonstrate extensive necrosis, with only small peripheral areas of mononuclear viable cells remaining, which may be confused with PSTT/ETT. In such cases, interpretation should rely on clinical findings since both immunostaining and molecular studies may be non-diagnostic.

In some cases histological findings may be iatrogenic and represent residual PSTT/ETT-like mononuclear areas of CC following chemotherapy (42,54). However, other tumours may have genuine apparent mixed PSTT/ETT and CC morphology in the uterus but CC-like appearance in distant metastases, and yet other tumours have been shown to display areas of PSTT juxtaposed with areas of CC (39, 43). Such cases may also demonstrate variable immunostaining) in the different areas with markers such as SALL4 (21). Similarly, clear distinction of PSTT and ETT may not be possible, and indeed, in one of the seminal publications describing ETT, some illustrated cases had areas of apparent PSTT-like morphology (38). It is, therefore, possible that CC and PSTT/ETT may arise from similar progenitor cells, with differentiation pathways dependent on environmental or other factors, including chemotherapy which may select chemoresistant clones. Finally, tumours with PSTT and ETT morphology may be associated with peripheral areas of PSN (see above)

<A>Issues in differential diagnoses of tumours and-tumour like conditions

<B>PSTT vs EPSR in curettage specimens

In a curettage specimen, exuberant infiltration of myometrium by hyperchromatic and pleomorphic trophoblastic cells with dense eosinophilic cytoplasm, bizarre multinucleate cells and vascular invasion by implantation site intermediate trophoblast (ISIT) may be seen with PSTT and EPSR (Figure 3 vs Figure 7). This is a challenging diagnosis especially if the material is scanty. Presence of mass lesion, absence of chorionic villi,

confluent growth of monomorphic cells with 'splitting' of myometrium, mitoses and elevated Ki67 (>1%) index are suggestive of PSTT even in limited material (47).

<B>CC vs pleomorphic trophoblast in the absence of chorionic villi

Pleomorphic trophoblast, either villous, extravillous, or within the implantation site, is a common feature of CHM lacking prognostic significance (56). The most problematic scenario is the finding of blood clot and fragments of pleomorphic trophoblast without associated chorionic villi, or other specific features, in a curettage specimen. In such cases, extensive sampling to look for molar chorionic villi is helpful (Figure 10 A- B). If no villi are seen and there is a recent history of CHM; it is most likely that such trophoblast derives from a retained CHM. Therefore, such specimens should be reported as 'atypical trophoblast' after complete inclusion of material and levels have been performed, with further clinical and biochemical surveillance being indicated. For practical purposes, in early pregnancy specimens, presence of chorionic villi in curettings excludes the diagnosis of CC. The diagnosis of CC should be reserved for cases in which there is proliferation of biphasic, highly atypical, trophoblast in the absence of chorionic villi, in conjunction with other abnormal clinical (invasion, mass lesion, metastases) and biochemical (highly raised serum hCG levels) features (57). The only exception to this approach an intraplacental CC.

<B>Early gestation trophoblast shell vs CC

Normal trophoblast of an early gestation shows biphasic appearance comprising mononuclear trophoblastic and syncytiotrophoblastic cells somewhat resembling CC.

However, although the cells are proliferative, they are smaller, more uniform, and not atypical and there are ordered differentiation. The trophoblast is present only in small quantity and may show chorionic villi (Figure 10 C- D). Presence of larger amounts of trophoblast, invasion and destruction of adjacent tissue is suspicious for choriocarcinoma.

PSN vs ETT

In a curettage specimen differentiating between PSN and ETT may be difficult. The finding of mass lesion, irregular border, mitotic activity and ki67> 10% should favour a diagnosis of ETT over PSN. IHC profile for both the lesions is similar but proliferation markers are usually helpful. Interpretation of the histologic findings in these scenarios must always be in the appropriate clinical, imaging and biochemical context, ideally as part of a multidisciplinary team approach.

ETT vs SCC

Both SCC and ETT are tumours of young reproductive age females and over 50% ETT's arise in the cervix and lower uterine segment. The tumour may involve the mucosal surface mimicking high grade CIN and hyaline material may be mistaken for keratin as a diagnostic pitfall. Presence of decidualised stromal cells, calcification and expression of trophoblastic markers HLA-G, inhibin, MelCAM, hCG and hPL may be helpful. Of note. although p16 is used as a surrogate marker of high-risk HPV in cervical squamous neoplasia, trophoblastic proliferations may stain although not diffusely and strongly for this marker. P63 is also positive in both these lesions. Thus, caution should

be exercised and used a panel of antibodies (58). HPV-ISH is helpful in establishing the diagnosis of SCC.

PSTT vs epitheloid leiomyosarcoma (LMS)

Epithelioid leiomyosarcoma may be confused with PSTT/ETT but finding spindle morphology as well as positivity for smooth muscle markers but negative staining for HLAG will aid in the diagnosis.

GTT vs Non-gestational tumours with trophoblastic phenotype

This has been discussed in detail in the article on Molecular genotyping (by Fisher, elsewhere in this issue) and it is well known that a range of poorly differentiated carcinomas have been reported to clinically and morphologically overlap with GTT, including expression of Hcg. Definitive diagnosis can be achieved using molecular methods as described above but often immunostains as well as generous sampling is helpful in establishing the final diagnosis.

The other uncommon differentials include PSTT Vs poorly differentiated endometrial Ca, PSTT Vs PEComa/ metastatic melanoma PSTT Vs ETT. Appropriate immunocytochemistry is helpful in these situations.

Future objectives

Although most women with a new diagnosis of GTT are cured with existing chemotherapy, 0·5–5·0% die as a result of multidrug resistance, necessitating novel approaches. Immunotherapy appears to be a promising candidate for these patients.

It's well recognised that PD-L1 is strongly expressed by gestational trophoblastic neoplasms, suggesting the ligand is involved in tumour–immune evasion. Trophoblast does not express the classical MHC-I molecules HLA-A and HLA-B, or MHC–II, offering protection from T-cell-mediated placental destruction. It is similarly negative for HLA-A and MHC-II. Our experience (59) has shown that strong expression of PD-L1 is not a biomarker of response to immunotherapy but density, and distribution of tumour-infiltrating lymphocytes and HLA-G expression correlates with anti-PD-1 response. The strong presence of infiltrating T cells in responders suggests pembrolizumab might activate HLA-C directed or indirect T cell cytotoxicity. Effectors other than classically restricted T-cells might also be relevant. One candidate is natural killer cells that express PD-1, are cytotoxic towards classical MHC-I- negative cells, and are inhibited by HLA-G, which additionally contributes to the maintenance of gestational tolerance through T-cell suppression. Further research in these areas may provide valuable insight into new treatment modalities guided by pathologists.

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Figure legends

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Figure 1. Haematoxylin and eosin staining of choriocarcinomas. Tumour is composed by bi/tri-phasic pleomorphic population of mononuclear and multinucleate cells with areas of haemorrhage and necrosis. (A,B). Atpia (C) and abundant mitotic activity (D) is noted.

Figure 2. Immunostaining of trophoblastic markers in choriocarcinoma (40X). The tumour is diffusely positive for CK18 (A), GATA-3 (B), HCG (C) and CD10 (D). Patchy positivity is seen with Inhibin (D) and MUC-4 (E). Very occasional cell is positive for P63 (F). Ki-67 proliferation index is high.

Figure 3. Haematoxylin and eosin staining of Placental site trophoblastic tumour. Diffuse and infiltrative growth of monomorphic intermediate trophoblast arranged in sheets and nests (A) separating myometrial fibres ("splitting") (B) and replacing uterine vessel walls with extensive fibrinoid deposition (C&D), reminiscent of the normal implantation site. Destructive vascular invasion with haemorrhage is much less frequent than that seen in CC.

Figure 4. Immunostaining of trophoblastic markers in (40X) Placental site trophoblastic tumour. Diffuse staining is seen for CK18 (A), Inhibin (B), HPL (C), Mel-CAM (D) and CD10 (E). Only occasional cells/ no staining seen for PLAP (F), P63 (G) and a few cells are positive for HCG (H)

Figure 5. Haematoxylin and eosin staining of Epitheloid trophoblastic tumour (ETT). Expansile circumscribed tumour with mononucleater trophoblast amidst hyalinised eosinophilic material and dystrophic calcification (A & B). This closely mimics squamous cell carcinoma . A peritumoral lymphocytic infiltrate is seen (C&D).

Figure 6 Haematoxylin and eosin staining of a microscopic Epitheloid trophoblastic tumour (ETT) in a patient under follow-up for APSN. Patient was symptomatic 31 months after initial diagnosis of APSN. No tumour was seen on imaging. Hysterectomy showed plaque like growth of monomorphic intermediate trophoblast on both sides of endometrial canal with associated dystrophic calcification.

Atypia, focal necrosis and mitoses were seen (1-2 / 10 HPFs). Superficial myometrial invasion is present on one side (C& D).

Figure 7 Haematoxylin and eosin staining of Exaggerated placental site reaction (EPSR). Superficial myometrium is invaded by individual cells and nests of intermediate trophoblast with organisation and morphological characteristics of normal implantation site (A). Implantation site-type multinucleate trophoblast giant cells (B& D) and vascular invasion (endovascular plugging) can appear alarming (C). Double Mel-CAM/ Ki-67 staining (E & F) shows virtually no proliferation within Mel-CAM lined trophoblast. In Figure E- Mel-CAM (red membranous) and Ki-67 (brown nuclear) while reverse pattern is seen in Figure F.

Figure 8 Haematoxylin and eosin staining of a Placental site nodule (PSN). Small (<5mm), circumscribed, paucicellular hyalinised nodules composed of intermediate trophoblast (A & B) lesion with minimal cytological atypia and no mitotic activity. Lesional cells are strongly positive for p63 (inset with low Ki-67 labelling index <5%).

Figure 9 Haematoxylin and eosin staining of an Atypical placental site nodule—(A). Circumscribed nodule with higher cellularity (B and C). Cohesive nests and cords with cytological and nuclear atypia (D). Necrosis within centre of nodule (inset with Ki-67 labelling index >5)

Figure 10 Mimics of Choriocarcinoma. A&B- Invasive hydatidiform mole. Extensive sheets of atypical and pleomorphic trophoblast was seen within myometrium and blood vessels with scanty molar villi identified on extensive sampling. Diagnosis of CC should be

reserved for cases with absence of chorionic villi in conjunction with abnormal clinical (invasion, mass lesion, metastases) and biochemical (highly raised serum hCG levels) features

C& D- Trophoblast of an early gestation (Trophoblastic shell) shows biphasic appearance comprising mononuclear trophoblastic and syncytiotrophoblastic cells resembling CC. Although proliferative, the cells are smaller, uniform, and show ordered differentiation.